A COMPARISON OF THE EFFECTS OF GOITROGENS ON THYROID ACTIVITY IN TRITURUS VIRIDESCENS AND DESMOGNATHUS FUSCUS [1]

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Salamanders of the genus *Triturus* have been used widely in studies of the histology, cytology, seasonal variation, and responses of the endocrine glands. Relatively few investigations of these matters have been made with members of other urodele genera. It would be interesting to know to what extent findings based on study of an exclusively aquatic newt are applicable to urodeles that are terrestrial in habit. There is some indication that the thyroid of *Triturus* may differ significantly in at least one respect from that of the terrestrial salamander, *Desmognathus*, namely, in the histological changes elicited by treatment with the goitrogenic drug, thiourea. Adams (1946) found that a high dosage and a long period of treatment are required to bring about hypertrophy and hyperplasia of the thyroid gland of *Triturus viridescens* whereas Fisher (1953) and Wheeler (1953) reported that the thyroid of *Desmognathus fuscus* responds much more readily and typically to this goitrogen. Since, however, the doses, environmental conditions, and length of treatment differed considerably in these studies, it is not possible to conclude with any assurance that *Triturus* is refractory to the effects of thiourea.

In our experiments a comparison is made of the responses of these two salamanders to treatment with two different goitrogens, thiourea and potassium perchlorate. The effects of these goitrogens on radioiodine uptake by the thyroid and on the histology of the gland were the basis for the comparison.

MATERIALS AND METHODS

Specimens of *Triturus (Diemyctylus) viridescens viridescens* (Rafinesque) were collected on October 20, 1955, from a pond near Monterey, Virginia. Specimens of *Desmognathus fuscus fuscus* (Rafinesque) were taken from beneath stones at the edge of a small stream on a thickly wooded hillside near Oliver Springs, Tennessee, over the period June 23–July 23, 1956. All animals were maintained in the laboratory for a minimum of two weeks before being subjected to experimental treatment. Both before and during treatment they were fed every other day, the *Triturus* with ground lean beef fortified with cod liver oil and calcium phosphate, and the *Desmognathus* with live meal-worm larvae. Throughout the

experimental period the specimens were kept in a constant-temperature room at $19.0° \pm 1.0°$ C.

Goitrogens were injected into the body cavity on alternate days. Some animals were given injections of 0.1 ml. of a 1.0% aqueous solution of thiourea; others received injections of 0.1 ml. of a 0.2% aqueous solution of potassium perchlorate. Controls were given injections of 0.1 ml. of distilled water. The injections were made with a 27-gauge needle introduced into the body cavity through the muscles at the base of the hind leg. We chose this site to prevent the loss of fluid that sometimes occurs when injections are made directly through the abdominal wall. Successive injections were always given on alternate sides.

The uptake and turnover of radioiodine in the thyroids of experimental and control animals were observed by the following method: The animal to be studied was injected intraperitoneally with 0.1 ml. of 10% Holtfreter's solution containing 50.0 μc/ml. of I[131]. At fixed intervals over a 2½-day period thereafter the animal was anesthetized in an aqueous solution of tricaine methane sulfonate (1 part in 1000) and the radioactivity of the thyroid and heart regions was measured by a scintillation counter consisting of a 1.5-inch NaI crystal cemented to the window of an RCA type-5819 photomultiplier tube and a conventional amplifier and binary scaler. The crystal and photomultiplier were mounted in a lead cylinder 5.2 cm. thick with a collimating slit measuring 4.0 by 12.0 mm. The ventrum of the anesthetized animal was apposed to the lead cylinder with the region to be counted directly over the slit.

The procedure just described was evolved from a series of preliminary experiments carried out to ascertain the optimal dosage of I[131] for reliable counts, the time required for maximum uptake by the thyroid, the extent of individual variation in uptake among control animals, and the possible effects of frequent anesthetization on the uptake. In one preliminary experiment, counts were made at eight successive levels on the anterior-posterior axes of I[131]-injected animals from the snout to the base of the tail, giving a profile of radioactivity. Relatively high counts were obtained in the region of the thyroid and in the abdomen. The heart region was selected as representative of the tissues in general (other than the gut) for comparison with the thyroid region. The counting on these two regions was done by centering the thyroid or the heart, as located with relation to external anatomical characters, over the collimating slit. During the experiment the mean background count was 1.68 counts per second. It did not vary significantly over the 60-hour counting period.

At the conclusion of the measurements of radioactivity the animals were killed. The lower jaws, containing the thyroid glands, were fixed in a solution containing equal parts of Bouin's solution and ethylene glycol monethyl ether. Sectioned thyroids were stained with Harris' hematoxylin and Ponceau de zylidine-orange II (Gray, 1952) for histological study.

The results to be described here were based on information obtained from the study of 72 salamanders divided into two series, one made up of animals that received injections for 30 days and one of animals that had 46 days of treatment. Each series consisted of six groups of six animals each; one group of control, one of thiourea-treated, and one of perchlorate-treated specimens for each of the two species.

Results

### 1. Effects of thiourea and perchlorate treatment upon the histology of the thyroid

The thyroid glands of the control animals of the 30- and 46-day series did not differ significantly and will be described together. The thyroids of ten *Desmognathus* appeared to have moderate secretory activity. The follicles were relatively large, the colloid was homogeneous, and there was a moderate number of chromophobe droplets. The epithelium was cuboidal to low columnar. There was some but not a great deal of individual variation among the ten specimens. The extremes of variation in epithelial height, follicle size, and vacuolization of the colloid are shown in the photomicrographs (Figs. 1 and 2). The thyroids of ten *Triturus* controls presented a definite contrast with those just described. The follicles were larger, the epithelium much more flattened, and chromophobe droplets were either entirely absent or quite sparse. There was less individual variation in this group than in the *Desmognathus* controls and a single photomicrograph will suffice to illustrate the entire set (Fig. 3).

Examination of five pairs of thyroids of *Desmognathus* given treatment with thiourea for 30 days (15 injections) revealed a definite response indicated by a marked increase in height of the follicular epithelium, a folding of the follicle walls, a reduction in the amount of colloid present, and an increase in the number of chromophobe droplets. The thyroids of animals given this treatment for 46 days (23 injections) showed little difference from those just described except for a further reduction in the amount of colloid. A typical example is shown in Figure 4. The thyroids of *Triturus* given thiourea for 30 days or for 46 days were almost precisely like those of controls. The only definite change is an indication of hyperemia in the glands, the sections showing enlarged capillaries and many more blood corpuscles than were seen in controls (Fig. 5).

Potassium perchlorate treatment produced a still greater effect than thiourea treatment in *Desmognathus*. Even after 30 days, intrafollicular colloid was almost entirely lacking and the increase in epithelial height was quite striking (Fig. 6). Thyroids of animals treated with perchlorate for 46 days showed no significant further change, perhaps indicating that a maximal response had already been elicited. In *Triturus*, perchlorate was no more effective than thiourea in causing histological changes in the thyroid. In fact, there was not even an indication of hyperemia in the glands of the perchlorate-treated specimens; the glands were indistinguishable from those of controls (Fig. 7).

2. Effect of goitrogens on uptake and release of I^{131}

Table I gives the compiled results of measurements of radioactivity in thyroids of the animals of the 46-day series in comparison with radioactivity in their heart regions during a 60-hour period after injection with I^{131}. As has been pointed out, the measurements made in the heart region are presumed to be representative of the non-thyroidal tissues in general, except the gut, where iodine was being concentrated and eliminated. The measurements were corrected for the physical decay of I^{131} that followed the injection, and an analysis of variance was made of the means of the corrected counts.
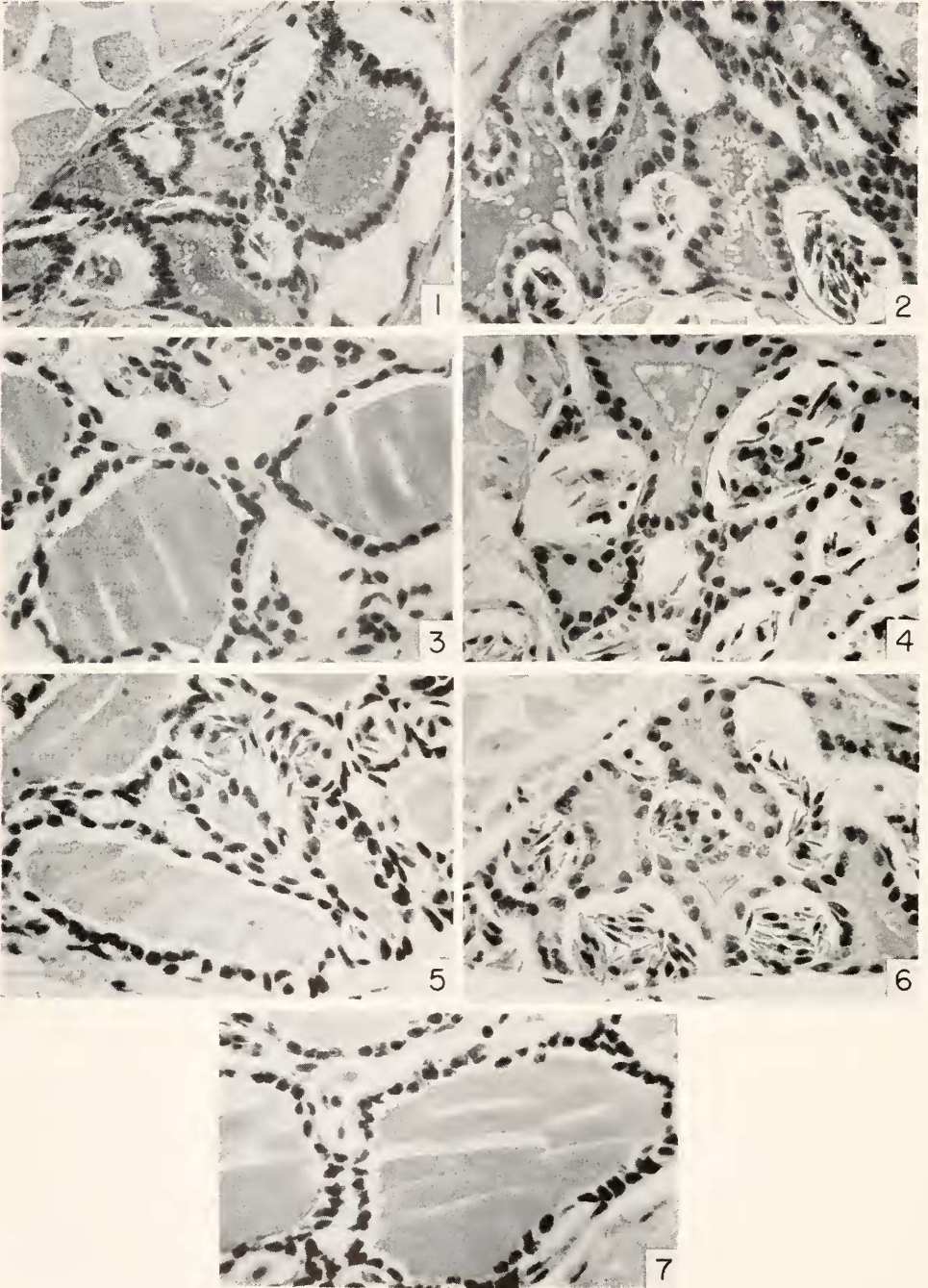
FIGURES 1–7.

The least significant difference beween any pair of means in Table I was found to be 2.63 at the 5% level. The interactions among these individual means are apparently of considerable physiological significance and will be interpreted in the following section of this paper. It is well to note also that the differences between the over-all mean counts for the two species, for the two types of treatment and control maneuvers, for the two locations of counting, and for the six periods of counting were all highly significant in the statistical sense, p being less than 0.1% in each instance.

Discussion

1. General comment and histological findings

The histology of the thyroid gland of *Triturus viridescens* and the seasonal changes it undergoes have been described by Morgan and Fales (1942) who found that the thyroid is moderately active in early winter, gradually increases in activity during mid-winter and spring (up to the breeding season) but has low activity during the summer months. Our *Triturus* controls had thyroids that agreed closely with their description of the summer thyroid characterized by flattened epithelial cells and abundant homogeneous colloid. No comparable study of seasonal varation is available for *Desmognathus*. Our findings showed, however, that there was a definite difference in the histology of the thyroid in these two salamanders at the same season and under the same temperature conditions, *Desmognathus* showing histological indications of a much higher level of activity than *Triturus*.

The experimental use of thiourea as a goitrogenic agent is now well known. Although the precise details of its mode of action are still not completely understood, it is generally accepted that it does not interfere with the ability of the thyroid gland to concentrate iodide but does inhibit its ability to utilize iodide for hormone synthesis (Pitt-Rivers, 1950; Roche and Michel, 1955). As a result of this inhibition, the level of thyroid hormone in the blood of animals treated with thiourea falls, causing increased production of thyroid-stimulating hormone (TSH) by the pituitary. In turn, the elevation of the TSH level induces hypertrophy, hyperplasia, and hyperemia in the thyroid and the release of its intrafollicular colloid. The "goitrogenic" effects of thiourea thus result from the pituitary stimulation rather than from the direct action of the drug itself. In our experiments, the 30- or 46-day treatments of *Desmognathus* with thiourea brought about all these structural changes in the thyroid. *Triturus*, similarly treated, showed no response

FIGURES 1 AND 2. Sections of thyroid glands from control specimens of *Desmognathus* killed in July. Histological evidence of moderate secretory activity ranges from the condition shown in Figure 1 to that shown in Figure 2.

FIGURE 3. Thyroids of *Triturus* killed in July gave a uniform appearance of inactivity as illustrated in this section.

FIGURE 4. Section of thyroid gland from *Desmognathus* treated with thiourea. Marked secretory activity is indicated.

FIGURE 5. Thyroid section from *Triturus* treated with thiourea.

FIGURES 6 AND 7. Thyroid sections from *Desmognathus* (6) and *Triturus* (7) treated with potassium perchlorate. Note marked response shown in *Desmognathus* and lack of response in *Triturus*.

aside from an increase in the vascularity of the gland. This lack of response in *Triturus* agrees with the findings of Adams (1946) who immersed specimens of *Triturus viridescens* in thiourea solutions that were increased in strength over a period of 42 days to 0.528% and then allowed the animals to remain in this concentration for 44 days longer. Such animals showed no change in thyroid histology as compared with controls. Adams found that animals treated similarly with solutions of twice this strength did show an increase in epithelial height and a reduction in colloid, indicating that a sufficiently high dosage may produce a response. Since, however, only two of the ten animals so treated survived to the end of the experiment, it seems clear that this concentration is quite toxic and the changes in thyroid structure may be related to this toxicity. Fisher (1953) reported histological changes in the thyroid of *Desmognathus* after thiourea administration that are in complete accord with our findings. His animals were treated by immersion in a 0.05% solution of thiourea and, although this is only one-tenth as strong as the weakest solution used by Adams, it caused hyperplasia, loss of colloid, and hyperemia—all of which became apparent after only two weeks and had increased markedly by six weeks. Although Fisher's experiments were performed in January and February and the season at which Adams' work was done is not given, the results of these two studies seemed to indicate, as did our findings, that the structure of the thyroid of *Triturus* was relatively unaffected by treatment with thiourea whereas that of *Desmognathus* was markedly altered.

The effects of perchlorate have apparently not been investigated previously for either of these animals. Studies on mammals indicate that the action of perchlorate is quite unlike that of thiourea, for it interferes with the process of iodide concentration (Wyngaarden, Wright and Ways, 1952). The thyroids of animals given effective doses of this drug are unable to accumulate iodine from the plasma. This, of course, prevents synthesis of thyroid hormone (TH) and, just as in thiourea treatment, the resultant lowering of the TH level and rise in TSH level cause the histological changes in the thyroid that are usually associated with a high rate of secretory activity. In our experiments the histological results of perchlorate treatment precisely paralleled those obtained with thiourea. The *Desmognathus* thyroid was strikingly affected but that of *Triturus* remained unchanged.

Desmognathus, then, after treatment with either of these two goitrogenic drugs, gives indication of the increased TSH production, which would be expected on the basis of the work with mammals. *Triturus* shows little or no sign of any increased TSH level. The lack of response in *Triturus* could be explained in at least two different ways. One possibility is that this salamander is refractory to the drugs so that, at the dosage level used, thiourea and perchlorate both failed to inhibit hormone synthesis. If this were the case, the treatment would have caused no lowering of the TH level in the blood; therefore, no stimulation of the pituitary to increased TSH production would have occurred. The other possibility is that the drugs were effective in preventing hormone synthesis but that the pituitary failed to respond in the usual way to the TH decrease. Then no increase in TSH would occur and none of the effects of this hormone on the thyroid would be observed. The results of the study of radioiodine uptake will serve to indicate which of these alternatives is correct.

2. Iodine uptake in control animals

It was observed (Table I) that in the *Desmognathus* controls the counts in the thyroid region were not significantly higher than those in the heart region at 6 hours after injection of I^{131}. By 12 hours, however, they had risen to a high level. They then declined steadily, reaching about one-half the peak level by 2.5 days, but remained significantly higher than the counts in the heart region throughout this period. *Triturus* controls, on the other hand, showed neither an initial difference in counts between thyroid and heart nor a steady decline in the counts of the thyroid region during the 2.5-day period, although the counts at the heart level declined steadily as I^{131} was eliminated from the body, with the result that the thyroid counts were significantly higher than the heart region counts from 24 hours on. Comparison of the thyroid counts with heart counts showed that radioiodine was taken up actively, reached a high level, and was rapidly released by the thyroid of *Desmognathus* whereas in *Triturus* the gland accumulated I^{131} more sluggishly and in lesser amounts and the turnover (during 2.5 days) was negligible. These results are in accord with the histological findings, which indicate a greater physiological activity of the thyroid in untreated *Desmognathus* than in untreated *Triturus*.

3. Iodine uptake in thiourea-treated animals

Table I shows that *Desmognathus* treated with thiourea had a high initial uptake of I^{131} by the thyroid (falling in the same range as the peak for the controls) and then a rapid loss; the differences between thyroid and heart regions were there-

TABLE I

Radioactivity (corrected for physical decay) in counts per second of specimens of Triturus viridescens and Desmognathus fuscus at the indicated periods of time after injection with 5 μc. of I^{131} after 46 days of treatment with goitrogens. Each number represents the mean of counts made on six specimens. The least significant difference (at the 5% level) calculated for these means is 2.63

Species	Time after I^{131} inj. hours	Control animals		Animals treated with thiourea		Animals treated with KClO₄	
		Region of thyroid	Region of heart	Region of thyroid	Region of heart	Region of thyroid	Region of heart
Triturus viridescens	6	10.30	10.94	9.64	9.96	10.25	11.68
	12	11.84	9.42	9.90	9.41	8.77	9.85
	24	11.70	8.59	8.90	8.80	5.96	6.22
	36	11.57	7.76	8.86	8.56	5.04	5.03
	48	11.14	7.47	8.79	8.06	3.92	4.06
	60	11.11	7.89	9.10	8.11	3.53	3.95
Desmognathus fuscus	6	8.68	6.80	10.22	6.84	6.40	6.95
	12	12.13	5.66	8.42	6.23	5.58	6.05
	24	10.98	4.56	6.39	4.13	4.67	4.43
	36	10.94	3.78	4.51	3.58	3.91	3.80
	48	8.73	3.64	4.15	3.16	3.14	3.33
	60	6.86	3.35	3.71	3.09	2.84	3.03

fore quite significant at 6 hours after injection of I[131] but not significant thereafter. In *Triturus,* similarly treated, the iodine content of the thyroid was different from that of the controls only in the 24- and 36-hour counts and the thyroid region showed no count significantly higher than that of the heart region at any time.

These results may be interpreted as follows. It is clear from the histological study that the thiourea treatment caused a hypertrophy of the thyroid in *Desmogna-thus* and one may assume that this is accompanied by a high affinity for iodine. This would account for the initial high uptake of I[131]. Since, however, the gland was unable to bind iodine, because of the treatment with thiourea, and also since the TSH level in the blood was presumably high, the I[131] that had been taken up was very rapidly lost. In *Triturus* the thyroid did not hypertrophy and therefore would not be expected to have a high affinity for iodine. Indeed, it takes up I[131] and retains it to about the same degree as the controls. Not being able to bind I[131] the thyroid releases it, but since the TSH level is apparently unaffected by treatment with thiourea in *Triturus,* the release of I[131] from the thyroid is much slower than in *Desmognathus.*

4. *Iodine uptake in animals treated with potassium perchlorate*

Treatment with perchlorate produced the same effect on radioiodine uptake in both salamanders. Counts in the thyroid region did not differ from those in the heart region at any time. Indeed, the counts over the thyroid were, in general, slightly lower than those over the heart, probably because of the difference in the volume of circulating blood in the two regions. In any case it seems clear that perchlorate was equally effective in these two animals in preventing any accumulation of I[131] by the thyroid.

In perchlorate-treated *Triturus,* the counts in the heart region declined more rapidly and reached a significantly lower level than in the control or thiourea-treated *Triturus.* This indicates that elimination of I[131] from the body goes on rapidly when iodide uptake by the thyroid is blocked but is in some way delayed when the thyroid is actively concentrating iodide. This delay occurs regardless of whether the iodine taken up by the thyroid is ultimately bound to protein. It is well known that, so long as there is no interference with the thyroid's ability to concentrate iodide, the gland acts as an iodine reservoir, continually taking up iodine from the plasma and passing it back at a slow but steady rate so that the decline in iodine content of the plasma caused by elimination from the body is partially compensated by the exchange of iodine with the thyroid. In perchlorate-treated animals, where no such mechanism is in operation, the rate of decline in the iodine content of the plasma would depend solely upon the rate of iodine excretion. It is noteworthy that the rate of decline in the heart-region counts in *Desmognathus* is essentially the same in all three groups. The failure of the thyroid to show any iodine reservoir effect in this animal, even when its iodide-concentrating ability is unimpaired, is probably to be ascribed to the high activity of the gland. In thiourea-treated *Desmognathus,* although the initial uptake of radioiodine was high, I[131] was very rapidly passed out to the plasma and, since none was bound, the decline in uptake in both thyroid and plasma was rapid, paralleling that of perchlorate-treated *Desmognathus,* where no iodide-concentration occurred at all. It may be assumed that in control *Desmognathus* any unbound

iodine is also rapidly exchanged with the plasma, and the counts in the heart region again fall rapidly. Undoubtedly, the counts in the thyroid region remain significantly high in the controls because in these animals, in contrast with the thiourea-treated specimens, a part of the iodine is bound to protein and is thus unavailable for ready passage into the plasma.

5. Possible adaptive significance of findings

The results of the study of radioiodine uptake indicate that the differences in histological response of the thyroid in *Triturus* and in *Desmognathus* should not be attributed to a difference in the direct effects of the goitrogens on thyroid functioning in the two. Perchlorate treatment definitely prevented thyroidal build-up of I^{131} in both, and there is good reason to conclude that thiourea treatment interfered with binding of I^{131} in both. It would seem, therefore, that the lack of histological change in the *Triturus* thyroid, as well as its normally inactive state, must be attributed to some unusual condition in the pituitary, involving a low level of TSH production. Such an explanation has been suggested to account for what is apparently a similar situation in the goldfish (*Carassius auratus*). The goldfish thyroid exhibits a histological appearance of very low activity throughout the year and at no season (Fortune, 1955) undergoes hypertrophy after administration of thiourea (or other goitrogens). It does, however, respond very strongly to injected TSH both histologically (Gorbman, 1940) and physiologically (Berg and Gorbman, 1954). Fortune (1956) suggests that the inactive thyroid of the goldfish (possibly acquired through many generations of artificial selection) may be an important adaptation permitting its tolerance of an exceptionally wide range of temperatures (0° to 41° C.) and cites the rise in thermal death point from 23° C. to 33° C. in the teleost *Phoxinus laevis* after treatment with thiourea as supporting evidence for his suggestion.

This hypothesis may also apply to *Triturus viridescens*. Although no specific data are available concerning the temperature range of this species, it is undoubtedly quite wide. The animal lives in pools and ponds in both wooded and open situations and has a geographical range from southeastern Canada to Georgia and Alabama. It is certainly able to survive successfully in ice-covered water, and the water temperature in unshaded ponds in mid-summer must frequently rise above 30° C. *Desmognathus,* on the other hand, lives in moist situations usually in close proximity to small streams in deep woods. In our experience it does not survive in the laboratory at temperatures above 25° C. and does best below 20° C. Several rather obvious devices for testing this theory come to mind. One, namely, observation of the effect of TSH administration on iodine metabolism in *Triturus,* is currently being employed by us.

Summary

1. Specimens of *Triturus viridescens* and *Desmognathus fuscus* were injected on alternate days with 0.1 ml. of 1.0% thiourea.   Others were injected on alternate

days with 0.1 ml. of 0.2% potassium perchlorate. Histological study was made of the thyroid glands of both experimental and control animals after 30 days and 46 days of treatment. Measurements of uptake and turnover of injected I^{131} were made on the animals treated for 46 days.

2. Evidence was obtained from the histological observations and from the use of radioiodine to show that although the thyroids of control specimens of *Desmognathus* were physiologically active, those of *Triturus* controls were rather inactive.

3. Both thiourea and potassium perchlorate inhibited thyroidal function in *Desmognathus,* as evidenced by both histological changes and changes in radioiodine uptake.

4. In *Triturus,* thiourea brought about only a slight hyperemia and potassium perchlorate produced no detected histological change in the thyroid. Radiological measurements after the injection of I^{131}, however, indicated that the same physiological responses taking place in *Desmognathus* also occurred in *Triturus* but at a lower level of thyroidal function.

5. Measurements of radioactivity in the heart region demonstrated that iodine was readily excreted from all the specimens of *Desmognathus* and from the individuals of *Triturus* treated with potassium perchlorate. Elimination of iodine was relatively slow in the other specimens of *Triturus*.

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